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Insecticidal Activity of Lichens against the Maize Weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

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ABSTRACT

Extracts of *Letharia vulpina* (L.) Hue and *Peltigera rufescens* (Weiss) Humb. lichen species and two major lichen compounds (diffractaic and usnic acids), isolated from *Usnea longissima* Ach., were tested against adults of the maize weevil, *Sitophilus zeamais* Motschulsky under laboratory conditions at various concentrations (2.5, 5, 10, 20 mg.ml^{-1} for extracts and 1.25, 2.5, 5, 10 mg.ml^{-1} for major compounds) and at 24, 48, 72, and 96 h period. Results showed that both the extracts and the secondary metabolites of *U. longissima* had significant insecticidal effects on adults of *S. zeamais*. Mortality rate was the highest at 96 h period at the treatment of maximum concentration of extracts (20 mg.ml^{-1}) and compounds (10 mg.ml^{-1}). The mortality rates attained 96.97, 95.96, 96.97 and 76.77% for *L. vulpine, P. rufescens,* diffractaic and usnic acids, respectively. No mortality was found in the control. The present results suggest that the lichen extracts and the secondary metabolites can provide a good potential for the control of *S. zeamais* adults.

Key words: Insecticidal activity, Lichen, Secondary metabolites, *Sitophilus zeamais*.

INTRODUCTION

Maize weevil, *Sitophilus zeamais* Motschulsky is a common pest all over the world. It causes significant damage to harvested stored grains and drastically decreases yields. Insecticides are currently the tool for pest control on commercial farms. However, many chemicals are often unsuccessful when used against this pest because of its rapid develop of insecticidal resistance (Gillott, 2005).

Recently, researchers have been looking for new bioinsecticides as alternatives for chemical pesticides. Plant extracts have become one of these alternatives (Kim *et al*., 2003; Yildirim *et al*., 2005; Negahban *et al*., 2007; Ogendo *et al*., 2008; Pavela, 2010; Rattan, 2010 and Ebadollahi, 2011).

Lichens, organisms formed through symbiosis between fungi and algae and/or cyanobacteria, are significant insecticide sources among biological insecticides (Emmerich *et al*., 1993). At least 60 lichen species produce antibiotic substances and among them lichen acids, such as usnic and vulpinic acids that have powerful antibiotic effects against some bacteria (Galun, 1988). Furthermore, it was defined that the lichens *Letharia vulpina* (L.) Hue and *Vulpicida pinastri* (Scop.) J.-E. Mattsson had been used to kill wolves and foxes found in winter harmed herds in some countries of Europe and Scandinavians (Aslan *et al*., 1998). Lichens usually contain only one or two major toxic substances, often found in high concentrations. For example, fumarprotocetraric acid, the major toxic substance found in *Cetraria islandica*, (11% of the contents) (Gudjonsdottir and Ingolfsdottir, 1977). The other example is *Pertusaria alaianta* that contained up to 20% of a mixture of chloroxanthones as major toxic substances (Huneck and Hoefle, 1978).

Many studies have indicated that lichen metabolites have insecticidal effects as some lichens have antifeedant and lethal characteristic on insects (Emmerich *et al*., 1993; Bombuwala, 2001; Kathirgamanathar *et al*., 2006; Balaji *et al*., 2007; Cetin *et al*. 2008 and Silva *et al*., 2009.

The aim of the present study was to evaluate the insecticidal effect of the extracts of two lichen species; *L. vulpina* and *Peltigera rufescens* (Weiss) Humb in *in-vivo* conditions against adults of *S. zeamais.*

MATERIALS AND METHODS

Insects rearing

S. zeamais adults were collected from Tokat province, Turkey in a storage house. Maize grains were purchased from local market and stored in a freezer at -20 ^{o}C. Maize grains for *S. zeamais* was washed by tap water, dried and heated to prevent pre–infestation. Before their use in the experiments, *S. zeamais* adults were reared in laboratory at 25±1 °C, 64±5 R.H. and L: D=12 h: 12 h at the Department of Plant Protection, Atatürk University, Turkey. Obtained adults from the stock culture were stored in separate insect cages provided with maize grains. Tests were carried out under the abovementioned laboratory conditions.

Plant materials and extraction of lichen species

L. vulpina and *P. rufescens* were collected from Erzurum while *Usnea longissima* Ach. was collected from Trabzon province in Turkey. After collection, lichens were exposed to dry indoor conditions. All samples were identified and stored at the herbarium of Kazım Karabekir Education Faculty, Atatürk University-Erzurum, Turkey. Air-dried lichen samples were pulverized and extracted by Soxhlet extractor. Each sample (30 g) was extracted by distilled n-haxane, diethyl ether, acetone, and methanol solvents. 300 ml from each solvent was used for extraction. Extraction by n-haxane and diethyl ether solvents was maintained for two days at 25^{o}C, while extraction by acetone and methanol solvents was maintained for three days at 25^{o}C. As the result of extraction, solutions were put together and the solvent was evaporated by an evaporator. In this way, total lichen substances were obtained. Extraction of *L. vulpina* and *P. rufescens* yielded 7.50 and 6.62% (w/w) of lichen substances, respectively. The yields were based on dry materials of plant samples.

Isolation of lichen secondary metabolites

An air-dried sample of *U. longissima* (250 g) was extracted by 500 ml of diethyl ether using a Soxhlet apparatus at 40°C. The crude extract of lichen sample was filtered and stored at 4°C for 24 h to precipitate usnic acid (UA). The UA precipitates were collected and subjected to silica gel (70-230 mesh) column chromatography (CC) by eluting with a $CHCl_3$: *n*-hexane (8:2) solvent system. At the end of this process, 2.10 g of usnic acid were obtained by a yield 0.84% (w/w). After the usnic acid precipitates were removed, the solution was concentrated using an evaporator under reduced pressure. The extract (18.75 g) was subjected to CC using silica gel (70-230 mesh) eluting with $CHCl_3$:*n*-hexane (7:3, 7.5:2.5, 9:1 and 10:0) and $CHCl_3$:CH_3OH (9:1) solvent systems. Thus, 5.75 g of diffractaic acid were purified. Spectral data were previously reported by (Bayir *et al*., 2006 and Odabasoglu *et al*., 2006).

Preparation of the lichen extracts and compound solutions

200 mg total substances obtained from *L. vulpina* and *P. rufescens* were dissolved separately in 10 ml of 80% acetone solvent and so stock solution with a concentration of 20 mg.ml^{-1} was obtained for each one. The stock solution was diluted by 80% acetone solvent and solutions with the concentration of 10, 5, and 2.5 mg.ml^{-1} were prepared. One hundred mg of diffractaic acid and 100 mg of usnic acid were dissolved separately in 10 ml of 80% acetone solvent and so a stock solution, with the concentration of 10 mg.ml^{-1} was obtained for each one. The stock solution was diluted by 80% acetone solvent and solutions with concentration of 1.25, 2.5, and 5 mg.ml^{-1} were prepared.

Bioassays

Adults of *S. zeamais* insects were placed in Petri dishes (9 cm) to test the toxicity of the solutions against them. Each replicate consisted of 33 adults of *S. zeamais* and provided with maize grains. A dose of 0.8 ml of solution was used for each Petri dish. Initial tests were done to establish the appropriate dose and exposure time ranges. Amounts of solutions applied were 1.25, 2.5, 5, 10, and 20 mg.ml^{-1} in each Petri dish. After exposure, mortality of adults was determined at 24, 48, 72, and 96 h duration. Petri dish, applied with only 80% acetone solution, was used as a control. Three replicates were used for each combination of dose and exposure time. Insecticidal activity of the solution was expressed as mortality percent of adults.

Statistical analysis

Differences between the insecticidal activities of lichen extracts tested were determined according to analysis of variances (ANOVA) test by using the SPSS 15.0 software package. Duncan Test was used for comparison of means. LC_{50} values were calculated, following the method of Finney (1971). Probit analysis of concentration-mortality data was conducted to estimate the LC_{50} values and associated 95% confidence limits for each treatment (EPA Probit Analysis).

RESULTS AND DISCUSSION

Toxicity effects of the extracts of *L. vulpina* and *P. rufescens* and the two secondary metabolites (diffractaic and usnic acids), obtained from *U. longissima*, on adults of *S. zeamais* are summarized in tables (1 and 2). Results showed that the extracts and the secondary metabolites had strong insecticidal effects on adults of *S. zeamais* in comparison with the control (Figs. 1 - 4). Higher concentration and longer exposure time resulted to maximum toxicity on the adults. Mortality rates, 24, 48, 72, and 96 h post treatment with different concentrations of lichen extracts and secondary metabolites, are given in figures (3 and 4). Analysis of variances demonstrated that the effect on mortality rate of the adults was highly significant on the basis of concentration and exposure time (Tables 1 and 2).

Mortality rates of the adults of *S. zeamais*, 96 h post treatment with a maximum concentration, were determined as 96.97 and 95.96% for *P. rufescens* and *L. vulpina* extracts and 96.97 and 76.77% for diffractaic and usnic acids, respectively. However, no mortality was recorded in the control (Tables 1, 2, and Figures 3, 4).

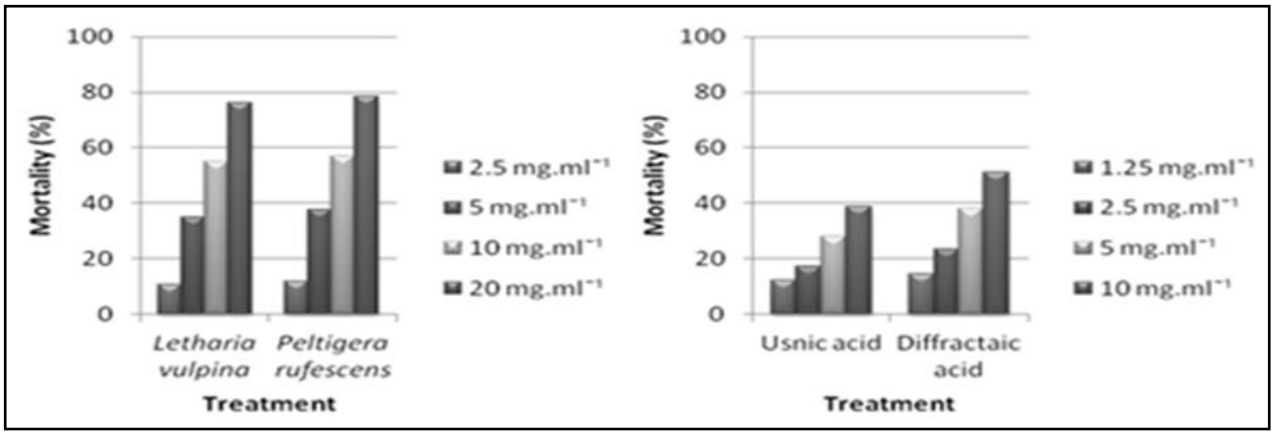

Fig. (1): Total mortality rates of adults of *Sitophilus zeamais* exposed to two lichen species extracts and secondary metabolites at different concentrations.

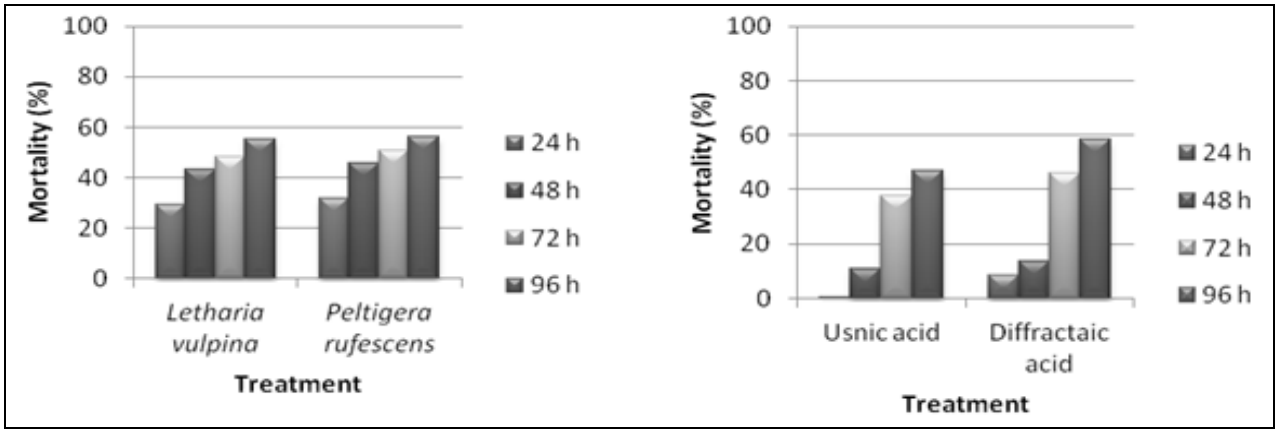

Fig. (2): Total mortality rates of adults of *Sitophilus zeamais* according to treatment time of two lichen species extracts and two lichen secondary metabolites.

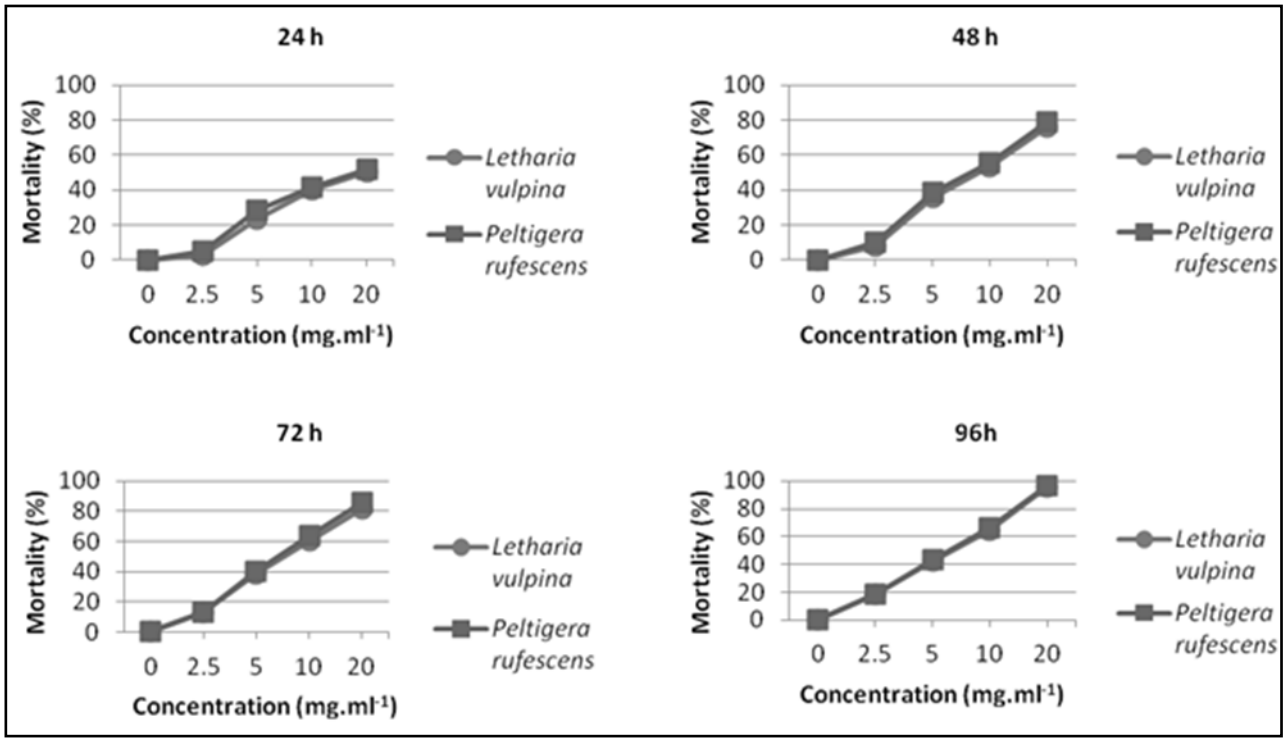

Fig. (3): Mortality rates in adults of *Sitophilus zeamais* in relation to exposure time and concentration of extract of two lichen species.

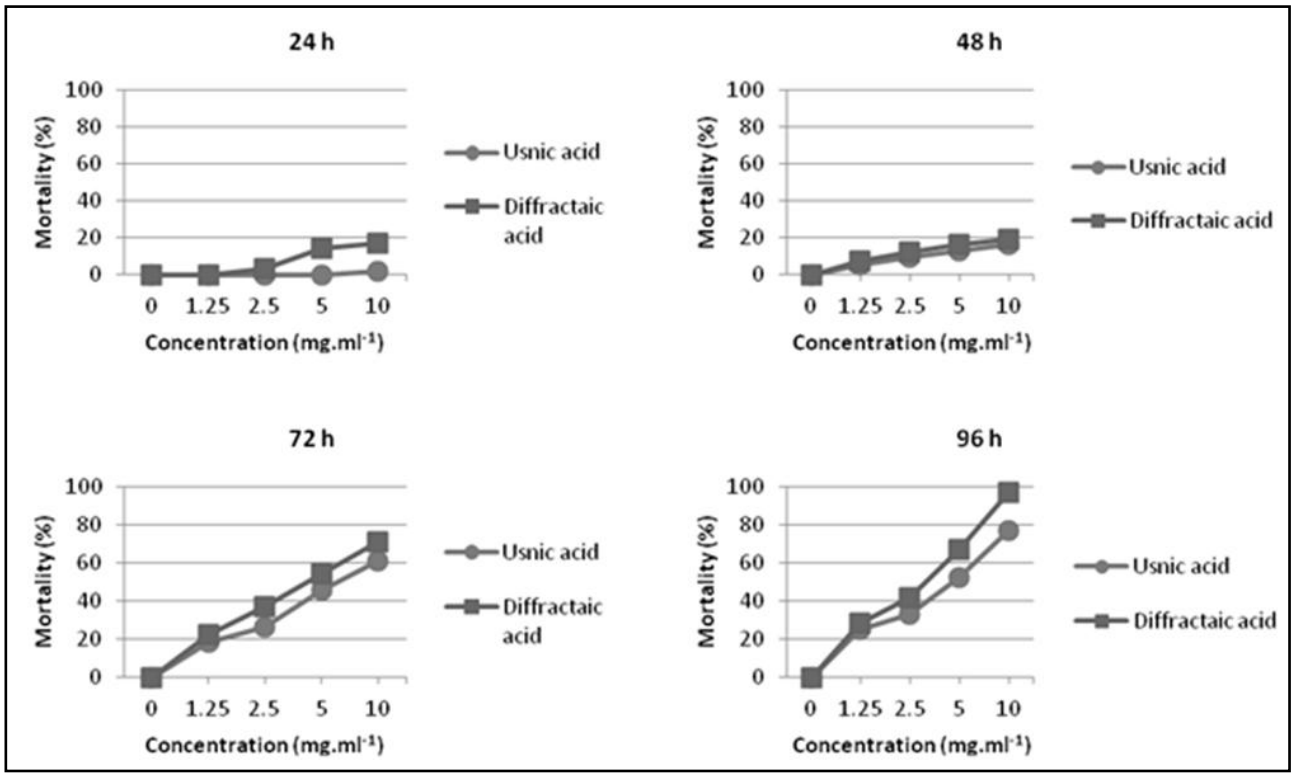

Fig. (4): Mortality adults of *Sitophilus zeamais* in relation to exposure time and concentration of two lichen secondary metabolites.

Table (1): Percent mortality effects of two lichen species on *Sitophilus zeamais* adults

Treatments	Concentration mg.ml^{-1}	Mean mortality (%)[a]			
		24[b]	48[b]	72[b]	96[b]
Letharia vulpina	2.5	3.03±0.58 a^{x}A^{y}	8.08±0.88 aAB	13.13±1.20 abBC	18.18±1.00 abC
	5	23.23±1.45 bA	35.35±1.67 bA	38.38±2.67 bcA	42.42±2.65 bcA
	10	40.40±0.67 cA	53.54±2.33 cA	60.61±4.51 cdA	64.65±3.84 cA
	20	50.51±1.67 cA	75.76±0.00 dB	81.82±1.15 dB	95.96±1.33 dC
Peltigera rufescens	2.5	5.05±0.88 aA	10.10±1.20 aAB	13.13±1.20 abAB	18.18±1.00 abB
	5	28.28±0.67 bA	38.38±3.71 bcA	40.40±3.33 bcA	43.43±2.96 bcA
	10	41.41±0.88 cA	55.56±1.67 cA	63.64±4.04 cdA	66.67±4.16 cA
	20	51.52±1.53 cA	78.79±0.58 dB	85.86±0.88 dB	96.97±1.00 dC
Control	–	0.00±0.00 aA	0.00±0.00 aA	0.00±0.00 aA	0.00±0.00 aA

Table (2): Percent mortality effects of two lichen secondary metabolites on *Sitophilus zeamais* adults

Treatments	Concentration (mg.ml^{-1})	Mean mortality(%)[a]			
		24[b]	48[b]	72[b]	96[b]
Usnic acid	1.25	0.00±0.00 a^{x}A^{y}	5.05±0.33 abB	18.18±0.58 bC	25.25±0.33 bD
	2.5	0.00±0.00 aA	9.09±0.58 bcdB	26.26±0.33 bC	33.33±0.58 cD
	5	0.00±0.00 aA	13.13±0.33 cdeB	45.45±0.58 cdC	52.53±0.33 eD
	10	2.02±0.67 aA	16.16±0.33 deB	60.61±0.58 efC	76.77±0.33 gD
Diffractaic acid	1.25	0.00±0.00 aA	7.07±0.33 bcB	22.22±0.67 bC	28.28±0.33 bcD
	2.5	3.03±0.58 aA	12.12±0.58 bcdeB	37.37±0.33 cC	41.41±0.88 dC
	5	14.14±0.33 bA	16.16±1.45 deA	54.55±1.15 deB	66.67±1.00 fC
	10	17.17±0.33 bA	19.19±0.88 eA	70.71±2.73 fB	96.97±0.58 hC
Control	–	0.00±0.00 aA	0.00±0.00 aA	0.00±0.00 aA	0.00±0.00 aA

[a] Mean±SE of three replicates, each set-up with 33 adults [b] Exposure time (h)

[x] Followed by the same lower case letter within a column are not significantly different at $p<0.05$

[y] Followed by the same capital letter at the same concentration (row) are not significantly different at $p<0.05$

Table (3): LC_{50} values ($mg.ml^{-1}$) of two lichen species extracts and two lichen secondary metabolites on adults of *Sitophilus zeamais*

Treatments	Exposure Time (h)	LC_{50} (Limits)	Slope (±SE) (Limits)
Letharia vulpina	72	7.461 (6.457–8.642)	2.189 (0.221) (1.756–2.622)
	96	5.953 (5.234–6.738)	2.639 (0.242) (2.165–3.113)
Peltigera rufescens	72	6.915 (6.028–7.922)	2.363 (0.227) (1.918–2.807)
	96	5.783 (5.098–6.525)	2.729 (0.247) (2.245–3.214)
Usnic acid	72	6.383 (5.044–8.889)	1.357 (0.204) (0.958–1.756)
	96	4.001 (3.298–4.928)	1.557 (0.203) (1.159–1.955)
Diffractaic acid	72	4.188 (3.410–5.263)	1.450 (0.201) (1.055–1.844)
	96	2.657 (2.292–3.044)	2.347 (0.233) (1.891–2.803)

Total mortality rate increased as the concentration increased. *P. rufescens* extract at the concentration of 20 $mg.ml^{-1}$ and diffractaic acid solution at 10 $mg.ml^{-1}$ showed highest insecticidal effects on the adults of *S. zeamais* (Fig. 1). Highest total mortality was obtained 96 h post exposure and highest adulticidal activity was reported for *P. rufescens* extract and diffractaic acid solution (Fig. 2).

LC_{50} values at 72 and 96 h were calculated (Table 3). The 96 h LC_{50} values were lower. Lichen extracts and lichen compounds showed more effect after 96 h of exposure. The 96 h LC_{50} values calculated for *P. rufescens* and *L. vulpina* extracts were 5.783 and 5.953 $mg.ml^{-1}$ and that calculated for usnic and diffractaic acids were 4.001 and 2.657 $mg.ml^{-1}$ for adults of *S. zeamais*, respectively. Low LC_{50} values (5.783 $mg.ml^{-1}$ for *P. rufescens* extract, 2.657 $mg.ml^{-1}$ for diffractaic acid) at 96 h indicated that the extract and the acid were highly toxic.

The present results showed that the extracts obtained from *L. vulpina*, *P. rufescens* and diffractaic and usnic acids from lichen secondary metabolites had varying degrees of adulticidal activities against adults of *S. zeamais*. Some studies demonstrated that, in general, the toxicity of extracts isolated from lichen samples against pests was related to their components (Emmerich *et al*., 1993; Bombuwala, 2001; Kathirgamanathar *et al*., 2006; Balaji *et al*., 2007; Cetin *et al*. 2008 and Silva *et al*., 2009). These results suggested that extracts isolated from different lichen species might have different toxicity levels, which can be attributed to their different chemical composition and different components (Sahip *et al.*, 2008).

In the present study, *P. rufescens* and *L. vulpina* extracts and diffractaic and usnic acids showed 96.97, 95.96, 96.97 and 76.77% mortality of adults. These results differ significantly at $p<0.05$ from other concentrations at 96 h. As well, significant disparities in mortalities (at $p<0.05$) occurred after different exposure times for the same concentration (Tables 1 and 2).

Natural products are now being considered as alternatives to the arsenal of synthetic compounds currently available (Dayan *et al*., 1999). Lichens are potential sources of natural compounds for pest management and are known as biological indicator organisms. They survive better in regions having unpolluted air, and produce secondary metabolites which are likely to be adaptive to the environment (Aslan *et al*., 1998). These metabolites are not harmful to the environment, but may have effect on the organisms that can be harmful for the lichens (i.e. phytophagous insects). For this reason, lichen acids isolated from lichen extracts are functional substances that are suitable to have effect only on the target organisms.

In conclusion, the effects of the extracts of the two lichen species and the two major lichen compounds tested were satisfactory as they had strong insecticidal activities against adults of *S. zeamais* and can be used as potential insecticidal materials.

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